

Canine amniotic membrane grafting in a dog affected with Bullous Keratopathy

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Bullous keratopathy (BK) is a nonspecific disease that leads to the formation of small vesicles in the epithelium and stroma of an oedematous cornea (Maggs, 2013). Successful surgical management of BK, and restoration of vision, by anterior stromal keratectomy followed by canine amniotic membrane (CAM) grafting is presented here.

A one and a half-year-old male Labrador Retriever was referred with redness in the right eye and protrusion of its anterior part. The animal has been suffering from the condition for the past three weeks. The condition, when presented, had been under treatment with antibiotic and hypertonic saline eye drops, oral antibiotics, and anti-inflammatory drugs, but with no response. Menace response on that eye was absent. Ophthalmoscopic examination revealed bullous transformation of majority of corneal surface, with blood-tinged accumulation beneath (Fig. 1A). The cornea was not transparent, except for a thin band near limbus. Fluoresceine dye test was found negative. Following application of topical proparacaine eye drops, punctate keratotomy was attempted to release the blood tinged fluid, but the corneal epithelium was found tough for the needle tip to create puncture. From the clinical presentation and findings, the condition was diagnosed as bullous keratopathy and anterior stromal keratectomy followed by CAM grafting was resorted to.

Allanto-amnion was collected aseptically, during a caesarean section. It was washed with sterile saline solution containing 0.1 mg/mL gentamicin and 2.5 mcg/mL amphotericin B (Kalpravidh *et al.*, 2009). The amnion was then harvested by gently peeling it off from the allantois, and spread onto a nitrocellulose paper with the stromal surface of the membrane facing the nitrocellulose paper and the epithelial side away. The nitrocellulose paper holding the amniotic membrane was then preserved in 98% glycerol at room temperature (Barros *et al.*, 2005).

The animal was premedicated with amoxicillin-sulbactam (20 mg/kg bost weight), pantoprazole (1 mg/kg), meloxicam (0.2 mg/kg) and atropine (0.04 mg/kg) administered intramuscularly. Anaesthesia was induced with xylazine, butorphanol and midazolam (0.2 mg/kg each), and tiletamine-zolazepam (2 mg/kg) taken in a single syringe and given intramuscularly. Anaesthesia was maintained using 1.0-1.25 % isoflurane in 100% oxygen through a

rebreathing circuit. The animal was positioned on lateral recumbency and the head was tilted using a positioner bag to position the affected eye against the surgeon. Eyelashes were trimmed, following which the cornea, conjunctiva and conjunctival fornix were irrigated and cleansed with diluted (1%) povidone-iodine. The eyeball was centrally positioned using stay sutures on bulbar conjunctiva. Anterior stromal keratectomy (Fig. 1B) was done to remove the ballooned corneal epithelium and the bullous stroma. The glycerol preserved canine amniotic membrane was rinsed with normal saline (Fig. 1C) and then grafted as an onlay graft (Fig. 1D) onto the corneal surface with the stromal surface of the amniotic membrane facing the corneal stroma. The graft was secured by suturing it to the perilimbal bulbar conjunctiva using 8-0 vicryl. The grafted cornea was then protected by a third eyelid flap. An Elizabethan collar was applied to prevent self-mutilation. Postoperatively, oral anti-inflammatory drug and antibiotic eye drops were then followed.

The operated eye was free of any discharges postoperatively. The third eyelid flap was released on the 15th postoperative day. The grafted membrane was not visible on gross examination. This could be attributed to the fact that the grafted amniotic membrane acted as a scaffold for cellular migration, epithelialisation and got incorporated to the host corneal tissue (Dua *et al.*, 2004). There was marked reduction in redness of the eye along with remarkable return of corneal transparency making the anterior chamber visible (Fig. 1E). Menace and photomotor light response were found positive. Ophthalmoscopic examination revealed intact corneal epithelial surface, but with mild corneal haziness. Fluoresceine dye test was negative which confirmed intact epithelium. This remarkable improvement in the corneal integrity following the grafting could be attributed to the inhibition of collagenases by the canine amniotic membrane (Barros *et al.*, 2005). Tobramycin eye drops were continued for two weeks. Review after two months revealed a completely clear and transparent cornea (Fig. 1F) with good visibility of the iris and posterior segment of the eye. Visual function tests were all found normal and the animal had regained vision. The gain in transparency of the cornea could be attributed to the anti-inflammatory, anti-angiogenic, epitheliotropic and anti-scarring properties of canine

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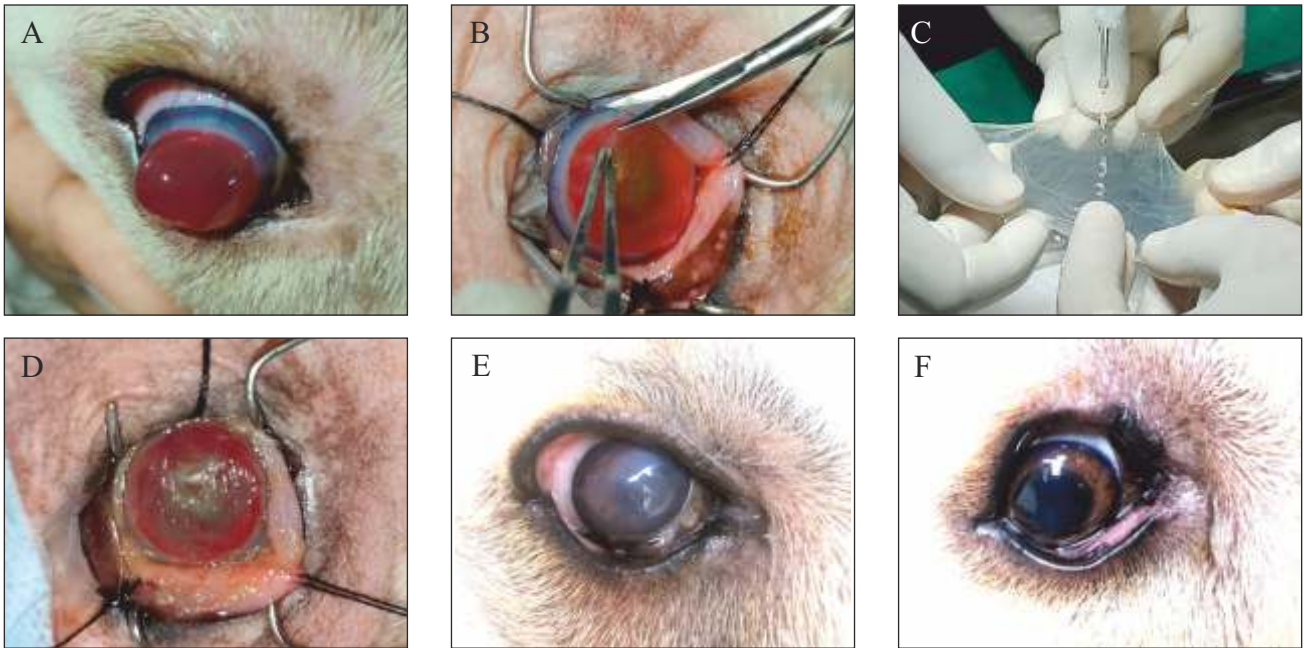


Fig. 1: (A) Bullous transformation of corneal surface, with blood-tinged accumulation beneath epithelium; (B) Anterior stromal keratectomy; (C) Preserved amniotic membrane was rinsed with normal saline before grafting; (D) Canine amniotic membrane grafted onto cornea as onlay graft; (E) Cornea on 15th day following CAM grafting; (F) Cornea after 2 months following CAM grafting.

amniotic membrane (Mary-John, 2023). It has also been reported earlier (Tseng *et al.*, 2004) that amniotic membrane is rarely associated with immunologic rejection, and hence plays a role in restoring the cornea to a more normal condition.

Maggs (2013) stated that unresponsive and progressive BK in dogs is best managed by thermokeratoplasty or superficial keratectomy followed by conjunctival grafting. Conjunctival grafting was not adopted in the present case owing to the corneal scarring that may result following healing with conjunctival grafting. Ori *et al.* (1994) had reported corneal scars when conjunctival pedicle grafts were used for treating corneal ulcers. Canine amniotic membrane grafting was adopted for corneal surface reconstruction in the current case of BK considering the earlier reports of successful healing of superficial to deep and extensive corneal ulcers in dogs when treated with decellularized processed human amniotic membrane (Nagashree *et al.*, 2020) and fresh preserved canine amniotic membrane (Mary John *et al.*, 2023) as onlay graft. The anti-inflammatory effect of CAM could be attributed to the reduced infiltration of polymorphonuclear leukocytes, expression of interleukin-10, interleukin-1 receptor antagonist and control of keratinocyte apoptosis. CAM exerts anti-scarring effect due to its anti-inflammatory properties and control of superfluous remodelling by inhibition of excessive fibroblast activation to myofibroblast. Findings from the present case of bullous keratopathy, suggest that canine amniotic membrane transplantation could be an efficient treatment option for symptomatic long-standing cases of BK with useful visual acuity in a low resource.

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